Morphological Attributes for Prediction of Quality of Made Tea During Early Selection Stages of Tea Breeding

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ABSTRACT

Quality of tea is evaluated by organoleptic analysis and chemical analysis of made tea. In the early stages of tea breeding and cultivar development process, the required amount of green leaf is not available for making processed tea to be subjected for organoleptic analysis in order to determine made tea quality. Therefore, the present study was carried out to evaluate the possibility of using certain morphological and growth characteristics as an early stage appraisal technique for made tea quality. Evaluations were made from ten cultivars with known quality for nine morphological characters. The resultant dendrogram of cluster analysis enabled separation of these cultivars into poor and good quality categories. Principal Components Analysis (PCA) revealed that, of the nine characters studied (eight quantitative and one qualitative), young leaf pubescence has the highest contribution towards clustering of cultivars into different quality groups. This study provides the base for rapid and early screening of base population of breeding material for made tea quality character and could be used as preliminary measure for identification of putative quality cultivars for further evaluation and testing in the latter stages of crop improvement program.

Keywords: Chemical analysis, made tea quality, morphological, organoleptic, pubescence

INTRODUCTION

Among the tea breeding strategies, breeding for made tea quality is one of the important considerations to attract high prices, mainly in the international tea market. Furthermore, production of quality tea is essential in Sri Lanka in order to sustain in the world tea market and to protect the name "Ceylon Tea" in the world tea trade. Therefore, breeding for quality is mandatory in crop improvement programs, apart from the yield.

Cup quality is an inherent character and one of the most important considerations in selecting potential commercial cultivars. According to previous studies, other than the genetic inheritance, various other factors such as climate/ seasonal changes (Agarwal, 1989; Owuor and Othieno, 1991; Botheju *et al.*,2000), agricultural practices (Dutta, 1960; Rahaman *et al.*,1978) and processing properties (Takeo, 1984; Agarwal, 1989; Owuor and Orchard, 1990a; Owuor and Orchard, 1990b; Owuor *et al.*,1992; Obanda and Owuor, 1993; Hajra,

2001) found to play a significant role in determining quality of the final product. It was also reported that the quality of harvested leaves (plucking standards) affects the quality and it had reflected in the tasters' evaluation too (Dutta, 1960; Basu and Choudhury, 1984).

Quality of tea is usually evaluated by organoleptic analysis and chemical analysis of made tea. Unlike other traits, selection of cultivars for better cup quality or made tea quality is complicated at the early stages of cultivar testing and evaluation program. As tea plant is an allogamous species, a large variation of characters occurs from bush to bush in existing seedling tea population and cross-pollinated progenies. Such variation is exploited through selection programs to develop new tea cultivars with enhanced traits. As the selection program advances from preliminary selection to the final, the variation among the test accessions decreases. Hence, selection of cultivars which also possess traits which could contribute to made tea quality also get eliminated during early stages of the evaluation programs, unless they possess attributes related to high yield. This is further aggravated by the absence of reliable quality testing methods using green leaf and the problems encountered in present organoleptic analysis for quality. Organoleptic or sensory evaluation is highly subjective and influenced by many factors which may not have direct relation to quality (Biswas *et al.*, 1971; Biswas *et al.*, 1973). Although the methods are available to process green leaf from a single bush (Lamb, 1939), the quantity is not adequate to a tea taster for an accurate estimation. To process green leaf into made tea using miniature processing system and to subject it for organoleptic analysis, a fair amount of green leaf (Owuor and Obanda, 1998), *i.e.*, about 500g of green leaf is necessary (A M T Amarakoon, personal communication). However, with the available number of plants per accession at early stages of evaluation trials, this requirement cannot be met.

As such, devising a method for selecting accession for inherent quality characters at single bush stage or progeny trial stage may overcome all the issues discussed above. Identification of chemical components in green leaf harvest will be one such possibility for estimating the inherent quality at this stage, although such a method is not available yet. Attempts were made to relate chemical composition in green leaf with organoleptic (sensory) evaluation and it has been observed correlation between these two factors was inconsistent (Owuor *et al.*, 1986; Obanda *et al.*, 1992; Owuor, 1996; Owuor and Obanda, 1998). Roberts and Fernando (1981) attempted to use the correlation between theaflavin and polyphenol content to the quality of tea as a criterion for clonal selection. They reported that the theaflavin content showed some correlation but it was not clear enough to be used as an absolute measure of quality. Herath *et al.* (1993) reported that there was no reliable correlation between the levels of flavanols and the quality of the cultivars studied. Accordingly, use of green leaf chemical parameters to predict made tea quality is not possible as yet.

Therefore, the present study investigates the possibility of using some morphological and growth attributes of tea plant to predict the quality of made tea at the early stages of tea crop improvement program.

MATERIALS AND METHODS

Ten recommended cultivars, representing high quality and poor quality character, which were previously evaluated and confirmed for their quality ratings (Anon. 2002) were used in this study (Table 1). Plants of those cultivars which were conserved in the *ex situ* field gene bank at the Tea Research Institute of Sri Lanka, Talawakelle were used to obtain the material. The plants were of the same age and have been maintained under similar growth conditions and following recommended cultural practices. Shoots comprising of mainly two leaves and apical bud were harvested from 20 bushes of each cultivar and samples of randomly collected 100g of harvested shoots were used for further analysis. The experiment was repeated at weekly intervals.

Table 1. Tea cultivars used for the study

High quality cultivars	**Poor quality cultivars**
N 2	DN *
DT 1	TRI 2025
TRI 777	TRI 2023
TRI 62/9	TRI 3013
TRI 4067	TRI 4052

*Rated as average quality (Kirtisinghe, 1968).

The following morphological and growth characters were used to score different cultivars in each category with a view of finding marker attributes to quality character:

1. Number of pubescence: Leaf discs with 0.5 cm diameter were made separately from first and second leaves. Pubescence counts were made using 3 randomly selected sample leaf discs per each leaf stage. Counts were made in the lower epidermis of the leaf discs under the light microscope at lower magnification (x 4).
2. Colour of young leaf: This was scored using the RHS colour chart for Plant tissues (R.H.S, 2007) and categorized according to the descriptors for tea developed by International Plant Genetic Resources Institute (IPGRI, 1997).
3. Following growth parameters were measured using randomly collected 5 samples that contain 100g of harvested two leaf and a bud:
   a. Total number of shoots in a 100g green leaf harvest
   b. Number of active shoots per 100g
   c. Number of dormant shoots per 100g
   d. Dry weight of 100 g of harvested shoots (g)

e. Length of terminal unfurled bud (cm)
f. Inter-node length between 1st and 2nd leaf (cm)

Data were analyzed statistically as a completely randomized design. The differences between mean values of each parameter were analyzed using Duncan's multiple range test (DMRT) and cluster diagram was constructed using average linkage method in Statistical Analysis Software package. A Principal Components Analysis was performed to determine the most contributory factors associated with grouping of cultivars in the dendrogram (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Mean values of the parameters measured are given in Table 2.

Table 2. Mean values of morphological and growth characters measured during the study

Cultivar	TS	AS	DS	SDW (g)	INL (cm)	BL (cm)	PB1	PB2	COL *
N 2	173^{f}	119^{b}	54^{g}	21.56^{ab}	2.64^{a}	2.56^{b}	222.4^{b}	170.4^{b}	2
DT 1	194^{e}	65^{e}	129^{d}	19.85^{c}	1.66^{bc}	2.46^{b}	205.6^{c}	147.4^{c}	2
TRI 777	220^{b}	108^{d}	112^{e}	23.85^{ab}	1.68^{bc}	2.46^{b}	124.0^{d}	136.4^{d}	2
TRI 62/9	189^{c}	41^{h}	148^{c}	23.64^{ab}	2.14^{a}	3.20^{a}	125.6^{d}	110.6^{e}	2
TRI 4067	224^{b}	57^{f}	167^{b}	21.56^{bc}	2.34^{a}	2.66^{b}	290.6^{a}	216.2^{a}	1
DN	188^{e}	115^{c}	73^{f}	24.08^{a}	1.70^{b}	2.70^{b}	103.2^{e}	71.6^{f}	1
TRI 2025	229^{a}	32^{i}	197^{a}	21.46^{ab}	1.32^{c}	2.56^{b}	5.8^{f}	7.4^{g}	2
TRI 2023	210^{c}	138^{a}	72^{f}	22.64^{ab}	1.42^{bc}	2.84^{b}	3.6^{f}	5.0^{gh}	2
TRI 3013	198^{d}	33^{i}	165^{b}	21.75^{bc}	1.86^{bc}	2.26^{b}	7.3^{f}	2.6^{h}	2
TRI 4052	193^{e}	47^{g}	146^{c}	22.64^{ab}	1.94^{b}	2.74^{b}	6.4^{f}	8.0^{gh}	2

Means with the same letter are not significantly different.

TS : Total number of shoots per 100g
AS : Number of active shoots per 100g
DS : Number of dormant shoots per 100g
SDW : Total shoot dry weight
INL : Internode length
BL : Terminal bud length
PB1 : Number of pubescence in the first leaf
PB2 : Number of pubescence in the second leaf
COL : Colour of young leaves
* Colour of young leaves : scored using RHS colour chart
1 - Green group N 137 A, 2 - Yellow – Green group 144 A

According to the results of the average linkage cluster analysis, cultivars subjected for the study were clustered into two main groups. The group 1 comprised with N 2, DT 1, TRI 4067, TRI 777, TRI 62/9 and DN. All those cultivars were considered as high quality cultivars except DN (Anon, 2002). In contrast, the 2nd group was comprised with non quality cultivars, TRI 2025, TRI 4052, TRI 2023 and TRI 3013 (Anon, 2002). Hence, the separation of high quality and poor quality cultivars into two main clusters is clearly evident in the dendrogram.

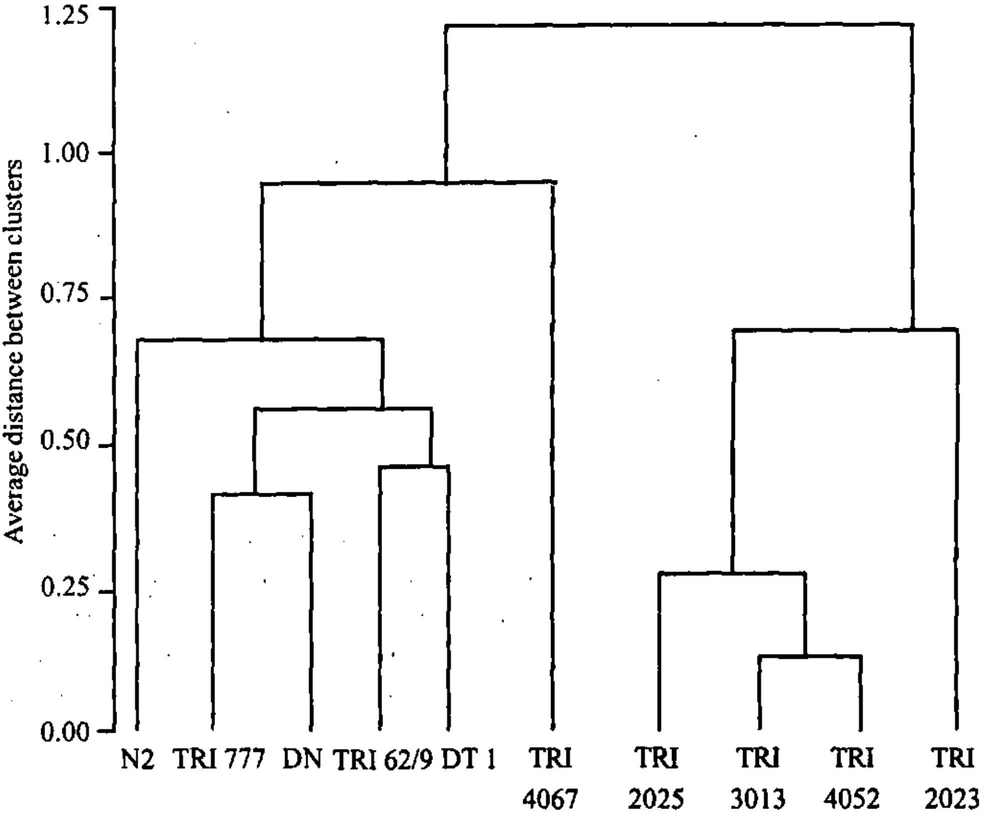

Figure 1. Dendrogram of average linkage cluster analysis based on morphological characteristics

Interestingly cultivar DN, which has categorized differently for quality, has been clustered with the cultivars belonging to high quality group in the present study. In the current list of recommended tea cultivars (Anon, 2002), DN was rated as a poor quality cultivar though in a previous study it was considered as an average quality cultivar (Kirtisinghe, 1968). Therefore, it is worthwhile re-considering the quality characteristics of DN to be able to support the present categorization.

The results of the Principal Components Analysis (PCA) on the morphological traits are presented in Table 3. It shows that the first three Principal Components (PCs) account for

Table 3. Eigenvalues, individual and cumulative percentage of variation explained by the first three principal components loadings of the seven quantitative characteristics.

Character	Principal Components		
	PC1	PC2	PC3
Number of active shoots	0.241	-0.625	-0.474
Number of dormant shoots	-0.275	0.602	-0.024
Internode length	0.464	0.168	0.364
Terminal bud length	-0.006	0.223	0.683
Number of pubescence in the first leaf	0.566	0.186	-0.102
Number of pubescence in the second leaf	0.567	0.178	-0.066
Total shoot dry weight	-0.106	0.321	0.619
Eigen value	2.76	2.02	1.64
Difference	0.74	0.38	
Variance explained (%)	39.47	28.93	23.43
Variance cumulative (%)	39.47	68.39	91.83

91% of the total variability 39%, 29% and 23% respectively (Table 3). According to the principal components loadings for characters, number of pubescence in the first and second leaf (0.57) show highest value indicating that they are the most contributing characters to the clustering order in the dendrogram. This suggests that the density of pubescence is highly correlated with the quality parameter of made tea. Internode length showed positive and equal loading of 0.46 with leaf pubescence. On the other hand second PC was correlated with characters such as number of active shoots and number of dormant shoots (0.6). Characters with high loadings for third PC were terminal bud length (0.7) and total shoot dry weight (0.6).

Large variation was observed in some parameters, i.e. pubescence in first leaf and second leaf, number of active and dormant shoots among the tested cultivars. Eigenvector values for leaf pubescence parameter was almost similar for first and second leaf, indicating that there is no difference in contribution to the clustering in the dendrogram based on the number of pubescence present in different leaf stages (first and second leaf). This further suggest that the number of pubescence present according to the leaf stage is not a contributory factor and hence counts made either in first or second leaf can be used for measuring pubescence to identify putative quality cultivars. The correlation coefficients among different morphological characteristics are given in Table 4. The significant correlation coefficient of 0.98 between number of pubescence present in the first leaf and the second leaf confirms the use of pubescent counts made either in first or second leaf for screening for quality at initial stages. There was a strong negative correlation (0.92) observed between number of active shoots and dormant shoots.

Table 4. Rank correlation coefficients between various morphological and growth characteristics

	AS	DS	INL	BL	PB1	PB2	SDW
AS	1.0000						
DS	-0.93	1.0000					
	(0.0001)						
INL	0.03	-0.18	1.0000				
	(0.9239)	(0.6176)					
BL	-0.29	0.21	0.41	1.0000			
	(0.4140)	(0.5524)	(0.2451)				
PB1	0.14	-0.18	0.69	-0.05	1.0000		
	(0.6903)	(0.6147)	(0.0280)	(0.8970)			
PB2	0.17	-0.18	0.68	0.01	0.98	1.0000	
	(0.0299)	(0.6327)	(0.6136)	(0.9831)	(<0.0001)		
SDW	0.26	-0.26	0.11	0.45	-0.35	-0.31	1.0000
	(0.7597)	(0.4714)	(0.4416)	(0.1955)	(0.3206)	(0.3842)	

Probability values for each correlation coefficient are indicated in parenthesis

AS : Number of active shoots per 100g

DS : Number of dormant shoots per 100g

INL : Internode length

BL : Terminal bud length

PB1 : Number of pubescence in the first leaf

PB2 : Number of pubescence in the second leaf

SDW : Total shoot dry weight

The use of different morphological parameters to identify tea cultivars with high made tea quality at early stages in breeding programs has been discussed by various authors. It was reported that the pubescence in young leaves and terminal bud had a positive correlation with better quality (Wight and Barua, 1954). A similar significant correlation between ordered arrangement of pubescence and the quality of orthodox tea in Assam was also reported by Wight and Gilchrist (1959). Weilian *et al.*, (1987) discussed an association between pubescence on the shoot and made tea quality. The work carried out in East Africa has shown that an increase in pubescence of the plants leads to small but significant improvement in the liquoring properties of the tea made in some seedling population (Green, 1967). Wu *et al.*, (1958) reported a correlation between dense pubescence and quality of black tea in Taiwan tea varieties and they further reported improvements in appearance of made tea produced from plants with high density of pubescence.

However, in previous studies they attempted to correlate presence or absence of pubescence in terminal buds with quality of made tea and the degree of pubescence were not considered.

In contrast, in the present study abundance of pubescence of first and second leaves was correlated with made tea quality. Therefore, the parametric approach used in the study could be further developed to build up multivariate models to predict made tea quality at early stages of the tea breeding program.

Colour of young leaves did not show wide variation among the cultivars tested and falls only into two categories of green group and yellow-green group. Colour of young leaves of cultivars DN and TRI 4067 were Green whereas other cultivars possess Yellow - Green colour young leaves. Weilian *et al.*, (1987) stated that made tea manufactured from the shoots with green or yellow-green colour is of good quality in most cases. Similar observations were noted by Venkataramani and Padmanabhan (1964) stating that the light green colour of the foliage and the pubescence are useful criteria in selecting for quality. Wight and Barua (1954) observed a genetically controlled occurrence of a red anthocyanin pigment in petiole but the quality of such varieties was found generally low and hence leaf colour was independent of the quality character. Green (1967) attempted to investigate the effect of leaf colour on liquoring properties and revealed that better tea is made from pale leaved plants than from dark leaved plants, although the difference is too small to warrant the use of leaf colour as a criterion for selection of cultivars for quality. In Kenya Taylor *et al.*, (1991) reported the relationship between black tea quality and plant pigment composition in green leaves and fitted a step-wise multiple regression model as a tool for predicting black tea quality at early stages of the breeding program. On the other hand Visser (1969) believed that a combination of slight pigmentation (anthocyanin) in the presence of pubescence influences quality in teas.

The use of chloroform test (Sanderson, 1963) in estimating fermentation rates of tea clones showed some influence on made tea quality (Samaraweera and Ranaweera, 1988). This method was also used in early selection of potential quality cultivars in tea breeding programs in Kenya (Seurei *et al.*, 1998) and in Pakistan (Waheed *et al.*, 2001). An association between fermentation rate and the quality character of the 10 cultivars tested in the present study was also revealed using the chloroform test (Data unpublished)

A review by Owuor and Obanda (1998) has suggested the possibility of using morphological features and various chemical constituents in selecting for quality at single bush level. In addition to morphological markers, attempts have been made by various researchers to correlate biochemical and chemical compounds in assessing the quality of made tea, although the correlation between those compounds and the quality was to be found inconsistent in many cases (Roberts and Fernando, 1981; Thanaraj and Seshadri, 1990; Owuor and Obanda, 1998) to be able to use them as chemical markers in screening for quality.

Recent developments in molecular marker technology have paved the way to search for a useful molecular marker for predicting the quality of tea during the early stage of tea breeding. Expression patterns of the basic genes related to accumulation of the catechins (major polyphenolic components conferring quality attributes in tea) and total polyphenols at different stages of tea leaf development and their relationship with catechin concentration were investigated by Mamati *et al.*, (2006) to determine potential role of these genes on the catechin accumulation patterns as potential markers in the early selection of tea breeding. However, the applicability of such techniques in tea breeding programs is yet to be discovered.

CONCLUSIONS

According to the results, pubescence in the lower epidermis of the first or second leaves can be used as a morphological marker to predict the quality of made tea at the early stages of the tea breeding programme. Leaf pubescence could be easily measured and hence could be used as a criterion in screening large number of progenies to identify the putative quality cultivars with reasonable accuracy for further confirmation with organoleptic evaluation in the latter stages of the cultivar evaluation program. It would also be possible to find out association between other morphological traits with made tea quality of such putative cultivars to develop comprehensive list of early selection indices for quality. Further investigations should be focused in developing more reliable chemical, biochemical and molecular markers as early selection criteria for quality in tea.

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